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14. ABSTRACT

Two experiments were performed to examine how different proteins in a carbohydrate-protein beverage affect postprandial amino acid (AA), glucose, and insulin responses. In one, volunteers drank 3 beverages in separate trials, each differing in protein type. Ten additional volunteers consumed the same drinks after 60 min of varying-intensity exercise. Blood glucose, insulin, glucose-dependent insulinotrophic polypeptide, and AAs were measured after consumption. Branched-chain AA concentrations peaked at 30 min and did not differ between beverages at rest or postexercise. There were no significant differences between beverages with respect to initial or total area under the curve for any outcome measures at rest or postexercise. High-carbohydrate beverages with various proportions of milk proteins from a supplier to the commercial industry had no impact on AA concentration. Retrospective chemical analysis of commercial proteins showed that casein was partially hydrolyzed; therefore, consumers should carefully consider the manufacturer or other factors when procuring these beverages for their purported physiological effects.

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Milk protein, exercise, carbohydrate supplements

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Plasma Amino Acid Responses After Consumption of Beverages With Varying Protein Type

Tracey J. Smith, Scott J. Montain, Danielle Anderson, and Andrew J. Young

Purpose: To examine how different proteins in a carbohydrate-protein beverage affect postprandial amino acid (AA), glucose, and insulin responses. ***Methods:*** Two randomized, repeated-measures experiments were performed. In one, 10 volunteers drank 3 carbohydrate-protein beverages (380 kcal, 76 g carbohydrate, 19 g protein, 2 g fat) in separate (>7 days) trials, each differing in protein type. All drinks consisted of cocoa (4 g) and nonfat dry milk (1 g) supplemented with casein (CAS), whey (WP), or a casein and whey blend (CAS-WP). Ten additional volunteers consumed the same drinks after 60 min of varying-intensity exercise (60% and 85% VO_{2peak}). Blood glucose, insulin, glucose-dependent insulinotrophic polypeptide (GIP), and AAs were measured every 15–30 min for 4 hr after beverage consumption. ***Results:*** Branched-chain AA concentrations peaked at 30 min and did not differ between beverages at rest (0.69 ± 0.12 mmol/L) or postexercise (0.70 ± 0.07 mmol/L). There were no significant differences between beverages with respect to initial (time 0–60) or total area under the curve (time 0–240) for any outcome measures at rest or postexercise. ***Conclusion:*** High-carbohydrate beverages containing various proportions of milk proteins procured from a supplier to the commercial industry had no impact on AA concentration. Retrospective chemical analysis of commercial proteins showed that casein was partially hydrolyzed; therefore, consumers should carefully consider the manufacturer (to ensure that the product contains intact protein) or other factors (i.e., cost or taste) when procuring these beverages for their purported physiological effects.

Keywords: milk protein, exercise, carbohydrate supplements

Consuming a carbohydrate- and protein-rich meal after aerobic exercise facilitates protein synthesis and attenuates proteolysis (Tipton & Wolfe, 1998); hence, postexercise "recovery" beverages have become popular. Evidence suggests that consuming different types of protein elicits subsequent changes in plasma amino acids (AA) and net protein turnover (Boirie et al., 1997; Dangin, Boirie, Guillet, & Beaufrere, 2002), but it remains unclear whether these differences persist when

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different proteins are incorporated into carbohydrate-protein beverages. Moreover, it is unclear whether a carbohydrate beverage containing a blend of isolated milk proteins (i.e., casein and whey) in a combination similar to milk can reproduce the unique AA-absorption pattern of milk.

Postprandial AA and hormone responses are modulated by the digestion rate of milk proteins, which differs depending on the physical state of the protein (i.e., intact vs. hydrolyzed). When intact casein (which is released slowly from the stomach) and whey (which is emptied rapidly from the stomach) are compared, whey produces a faster, transient increase in plasma AA concentration, whereas casein results in a slower but more prolonged increase in plasma AA concentrations (Boirie et al., 1997; Dangin et al., 2001). When casein's chain length is shortened by hydrolyzation, however, the casein peptides are digested more quickly, which produces a rapid postprandial AA response similar to the AA profile elicited by whey and enhances insulin secretion (Calbet & MacLean, 2002; Dangin et al., 2001; Silk et al., 1979; van Loon, Saris, Verhagen, & Wagenmakers, 2000). Milk, which is composed of 80% casein and 20% whey, produces a plasma AA profile that captures the rapid but transient response attributable to whey protein, as well as the more sustained increase associated with intact casein (Lacroix et al., 2006; Nilsson, Stenberg, Frid, Holst, & Bjorck, 2004).

The different AA responses elicited after consuming casein or whey proteins, alone or in combination, alter protein synthesis and protein breakdown (Bos et al., 1999; Dangin et al., 2002). Protein synthesis is stimulated more (68%) after consuming whey than intact casein. Consuming intact casein, however, markedly decreases protein breakdown (~30%) compared with whey (Dangin et al., 2002). Although whey protein is efficient at stimulating protein synthesis, it is oxidized more quickly than casein. Thus, a meal of intact casein (a "slow" protein) results in a more prolonged net leucine balance than consuming whey (a "fast" protein) when administered at rest (Dangin et al., 2002). The combination of casein and whey seems optimal, because milk's net postprandial protein utilization is higher than that of casein or whey protein alone (Bos et al.).

Consuming carbohydrate with protein slows protein digestion and absorption as a result of delayed gastric emptying (Fouillet et al., 2001; Gaudichon et al., 1999; Mariotti, Mahe, Luengo, Benamouzig, & Tome, 2000) and attenuates the AA response compared with consumption of protein alone (Dangin et al., 2002). The unique plasma AA profile attributable to protein type is maintained, however, when the carbohydrate-to-protein ratio is less than 2:1 (Dangin et al., 2001, 2002; Lacroix et al., 2006; Nilsson et al., 2004). Postexercise recovery supplements or high-carbohydrate meal replacements typically have a carbohydrate-to-protein ratio of approximately 4:1; however, it is not known if the additional carbohydrates relative to protein interfere with the digestion and plasma AA response attributable to whey and casein after consumption.

The AA and hormonal responses produced by ingesting varying proportions of milk proteins have been studied in people at rest but not during postexercise recovery. It is well known that the stimulus of exercise changes the AA and hormonal milieu compared with resting conditions (Rennie & Tipton, 2000). There is some evidence that ingesting casein and whey protein after resistance exercise stimulates net muscle protein balance to a similar degree, despite differing AA blood profiles and insulin responses (Tipton et al., 2004). Whether the same is true after aerobic exercise has not been investigated. It is evident that the timing of

nutrient ingestion in the postexercise period affects muscle glycogen stores; that is, postexercise muscle glycogen resynthesis is enhanced when carbohydrates are ingested within the first hour and slowed if carbohydrate intake is either inadequate or delayed (Ivy, Katz, Cutler, Sherman, & Coyle, 1988). Thus, the absorption and utilization of a carbohydrate-protein beverage, with varying milk proteins, might be different if consumed at rest or at different times during recovery from exercise.

The primary purpose of this study was to investigate the insulinogenic and AA responses after ingestion of chocolate-flavored carbohydrate beverages (75 g carbohydrates in a 4:1 carbohydrate-to-protein ratio) containing differing proportions of casein and whey protein procured from a supplier to the commercial industry. The study was separated into two experiments: In one, beverages were ingested at rest; in the other, beverages were consumed immediately after a 60-min treadmill exercise bout. In each experiment, we compared the responses produced by beverages containing a blend of casein and whey protein (CAS-WP) with those produced by beverages matched for total energy, protein, carbohydrate, and fat but containing either predominantly casein (CAS) or whey protein (WP). We hypothesized that WP would produce a faster, transient increase in plasma AA concentration (than CAS and CAS-WP), and CAS would produce a lower, more prolonged increase in plasma AA concentration (than WP and CAS-WP). In regard to hormonal response, we hypothesized that WP would stimulate a higher response of insulin and glucose-dependent insulinotrophic polypeptide (GIP) than would CAS. Finally, we hypothesized that CAS-WP would demonstrate a modified AA and hormonal response similar to milk, independent of exercise.

Methods

Participants

Twenty healthy men gave informed, written consent to participate in this investigation after receiving an oral and written explanation of all study procedures and risks. The protocol for this research was reviewed and approved by institutional scientific and human subjects committees. All volunteers were medically cleared for participation in accordance with the U.S. Army Research Institute of Environmental Medicine's guidelines for human use. Volunteers who had had a recent weight gain or loss (>2.3 kg [5 lb]) in the past month, had an allergy to milk proteins, or had a disease or took medication known to affect energy metabolism, appetite, or physical exertion were excluded from study participation.

Experimental Design

Two randomized, double-blind, repeated-measures experiments were performed. In Experiment 1, 10 volunteers drank a carbohydrate-protein beverage in three separate trials (separated by at least 7 days); each trial employed beverages with varying amounts of casein and whey. Subsequently, volunteers ($n = 10$) in Experiment 2 consumed the same beverages after 60 min of treadmill exercise alternating between 60% (10 min) and 80% (5 min) VO_{2peak}. Outcome measures included body weight and blood levels of glucose, insulin, GIP, and AAs. Figure 1 depicts the specific activities carried out on each test day.

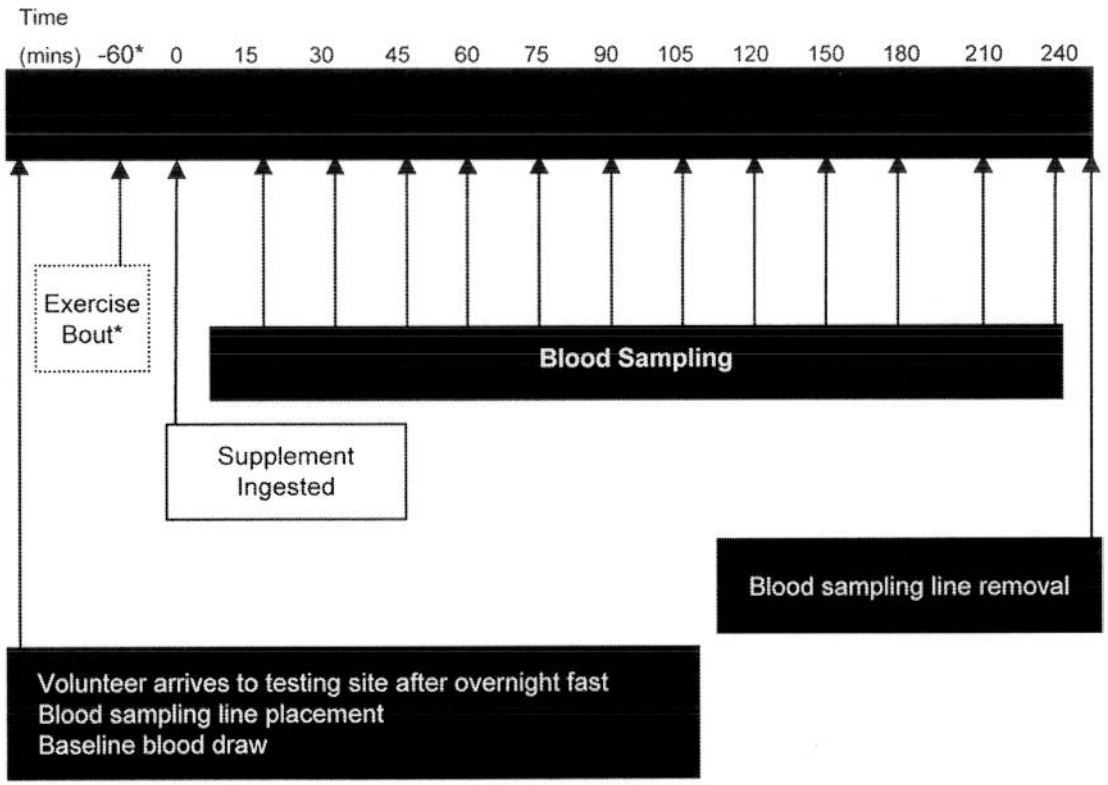

Figure 1 — Experimental design.

Anthropometrics

Height was measured at baseline, and body weight was measured at baseline and on the morning of each test day using a calibrated electronic battery-powered scale accurate to 0.1 kg.

Dietary Instruction

Before the study began, registered dietitians provided education on study dietary guidelines: (a) Maintain a consistent protein intake during the course of the study to stabilize hepatic enzymes, (b) refrain from all food and beverages (except water) after 10 p.m. the evening before each test session, and (c) ensure that macronutrient content of the evening meal (i.e., the last meal before testing) was consistent between all three test sessions. Compliance with these guidelines was documented on the morning of each test session via a 24-hr food recall.

Graded Exercise Test

For volunteers participating in the postexercise trial, peak oxygen uptake (VO_{2peak}) was determined by analysis of expired gases during treadmill exercise using indirect open-circuit spirometry (True Max 2400, Parvomedics, Sandy, UT). The exercise protocol was progressive in intensity, continuous in nature, and performed in a temperate (~20 °C) environment. Initially, volunteers ran for 4 min at 6.5 mph and 0% grade, after which the grade was increased 2% every 2 min until the volunteer reached subjective volitional exhaustion and signaled to stop the treadmill.

Dietary Intervention

Beverages were packaged in powder form and reconstituted with ~16 fl oz of water before ingestion. All beverages contained cocoa powder (4 g protein) and nonfat dry milk (1.5 g protein). Three protein powders (Fonterra, New Zealand) were used to formulate the carbohydrate-protein beverages. WP was made with Alacen 895, CAS was made with Alanate 391, and CAS-WP was made with a combination of Alapro 4850 and Alanate 391. Whey and casein, respectively, contributed the following percentages to total protein content: 70% and 9% (WP), 2% and 77% (CAS), and 13% and 66% (CAS-WP). Percentages of intact protein in WP, CAS, and CAS-WP, as determined by gel electrophoresis post hoc, were 94%, 57%, and 66%, respectively. Nutritional information (Table 1) and AA composition (Table 2) of the beverages were determined using computerized nutrient analysis (ESHA Genesis, Salem, OR) and information provided by the manufacturer (Fonterra, New Zealand).

Blood Sampling and Biochemical Analyses

An indwelling catheter was placed in the volunteer's forearm or antecubital space on arrival at the testing site. Baseline blood samples were taken after placement of the catheters (not more than 15 min before the initiation of the testing phase) and at various time points as indicated in Figure 1. Blood for serum was allowed to

Table 1 Nutritional Information per Serving Size (448 g/16 fl oz)

Nutrient	Amount
kcal	360
Calories from fat	21
Calories from saturated fat	2.0
Protein	19 g
Total carbohydrates	76 g
dietary fiber	5.7 g
soluble fiber	0.4 g
total sugar	50 g
lactose	5 g
sucrose	45 g
other carbohydrates (maltodextrin, cocoa powder)	19 g
Total fat	2 g
saturated fat	0.2 g
cholesterol	8.7 mg
Water	44 g

Table 2 Amino Acid Profile of Supplements (448 g/16 fl oz) From Manufacturer's Information

	Casein and whey	Whey	Casein
Essential amino acids			
isoleucine	0.57	0.73	0.56
leucine	1.07	1.66	0.98
lysine	0.92	1.32	0.91
methionine	0.31	0.28	0.31
phenylalanine	0.58	0.46	0.61
threonine	0.49	0.60	0.52
tryptophan	0.18	0.29	0.13
valine	0.69	0.64	0.68
Nonessential amino acids			
histidine	0.33	0.24	0.32
alanine	0.33	0.61	0.30
arginine	0.42	0.36	0.44
aspartic acid	0.80	1.45	0.76
cysteine	0.07	0.46	0.06
glutamic acid	2.41	2.07	2.42
glycine	0.18	0.18	0.17
proline	1.10	0.51	1.13
serine	0.56	0.50	0.59
tyrosine	0.61	0.51	0.63
Total (g)[a]	11.62	12.87	11.53

Note. Additional protein (5.5 g) was also contributed by the cocoa powder and nonfat dry milk.
[a]Additional protein contributed by amino acids that were not provided by the manufacturer.

coagulate for 30 min before being centrifuged for 10 min at 3,600 rpm. Resulting plasma and serum were frozen at –20 °C until analysis was performed at Pennington Biomedical Research Center (Baton Rouge, LA).

Plasma glucose concentrations were determined using Synchron CX7 (Beckman-Coulter, Brea, CA) using a glucose oxidase electrode. Plasma insulin concentrations were determined using immunoassays with fluorescence detection on an Immulite 2000 (DPC, Los Angeles, CA). Plasma nonesterified fatty-acid and glycerol concentrations were determined using a Synchron CX5 (Beckman-Coulter) with enzymatic reaction with colorimetric detection. Plasma GIP concentrations were analyzed with enzyme-linked immunosorbent assay. High-performance liquid chromatography (Agilent, Santa Clara, CA) was used to analyze plasma concentrations of threonine, serine, glutamine, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine (Terrlink, van Leeuwen, & Houdijk, 1994).

Calculations

Area under the curve (AUC) was calculated individually for each volunteer's three trials (i.e., three test drinks) for the following outcome measures: AAs, GIP, glucose, and insulin. Briefly, AUC with respect to ground (AUC_G; Matthews, Altman, Campbell, & Royston, 1990; Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003) represents the total AUC for all measurements with consideration for the time difference between measurements (t_i) and their distance from zero. In this equation, m_i represents the individual measurement and n equals the total number of measurements.

$$AUC_G = \sum_{i-1}^{n-1} \frac{(m_{i+1} + mi)t_i}{2}$$

Insulinogenic index, versus insulin alone, was used to distinguish between glucose-mediated insulin response and other possible insulin stimulators present in the beverages (Nilsson et al., 2004). In this equation, the AUC_G for insulin and glucose is calculated from time 0–30 min.

$$\frac{\text{insulin } AUC_G(T0-30)}{\text{glucose } AUC_G(T0-30)}$$

Statistics

Data from the rest and exercise trials were analyzed separately using SPSS statistical software (v. 15.0, Chicago). The Shapiro–Wilk test was used to examine normality of each variable. One-way repeated-measures analysis of variance was used to compare beverages in terms of the following outcome measures: energy and macronutrient intake before study days; calculated AUCs for AAs, glucose, insulin, and GIP; and insulinogenic index. Two-way repeated-measures analysis of variance was used to analyze drink-by-time differences between beverages in regard to blood concentrations of AA, glucose, insulin, and GIP—that is, time (min) to peak concentration and peak concentrations. Epsilon, using the Greenhouse–Geiser correction (when epsilon < .75) or Huynh–Feldt correction (if

Greenhouse–Geiser epsilon > .75), was used to adjust the degrees of freedom if Mauchly's test of sphericity significantly deviated from the assumption of sphericity. If a significant F ratio was observed for a main effect, post hoc comparisons were made using Tukey's honestly significant difference. Results are presented as $M \pm SD$. Statistical significance was set a priori at $p < .05$.

Results

Demographics

Baseline volunteer characteristics for rest ($n = 10$) and postexercise ($n = 10$) are presented in Table 3. Volunteers remained weight stable between the three beverage trials.

Diet Characteristics

Characteristics of dietary intake 1 day before each test day are presented in Table 4. Diet characteristics and duration of the overnight fast (10.2 ± 0.8 hr) did not significantly differ between the three beverage trials.

Table 3 Descriptive Statistics

	M	*SD*
Rest		
height (in.)	70	1
weight (kg)	73	4
age (years)	22	1
Postexercise		
height (in.)	69	1
weight (kg)	75	4
age (years)	21	1
VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{1}$)	51	1

Table 4 Diet Characteristics the Day Before Testing

	Rest		Postexercise	
	M	*SD*	*M*	*SD*
Energy (kcal)	2,663	572	2,545	827
Protein (g)	120	24	113	33
Protein (g/kg)	1.7	0.4	1.5	0.5
Carbohydrate (g)	290	102	315	115
Fat (g)	117	24	97	48

Glucose, Insulin, and GIP

Figures 2 and 3 present the postprandial responses for glucose, insulin, and GIP at rest and postexercise. Glucose, insulin, and GIP increased ($p < .001$) after ingestion of WP, CAS, and CAS-WP, at rest and postexercise. There was no significant beverage or beverage-by-time interaction for any outcome measure, however, regardless of whether the beverage was consumed at rest or postexercise.

At rest and postexercise, glucose reached peak concentration 15 min after beverage consumption, independent of beverage type. At rest, WP, CAS, and CAS-WP baseline glucose concentrations, with percent increase to peak in parentheses, were 86 ± 7 (44% ± 23%), 90 ± 7 (74% ± 36%), and 88 ± 4 mg/dl (77% ± 36%), respectively. Postexercise WP, CAS, and CAS-WP baseline glucose concentrations (with percent increase to peak) were 90 ± 6 (64% ± 38%), 87 ± 8 (67% ± 53%), and 87 ± 10 mg/dl (69% ± 34%), respectively.

At rest, insulin reached peak concentration in response to WP and CAS 15 min after beverage consumption, and to CAS-WP, 30 min after beverage consumption. Postexercise, insulin reached peak concentration in response to all beverages 15 min after beverage consumption. At rest, WP, CAS, and CAS-WP baseline insulin concentrations, with peak concentration in parentheses, were 2 ± 2 (45% ± 34%), 4 ± 3 (50% ± 32%), and 3 ± 3 mg/dl (79% ± 37%), respectively. Postexercise, WP, CAS, and CAS-WP baseline insulin concentrations, with peak concentration in parentheses, were 6 ± 3 (44% ± 20%), 6 ± 2 (60% ± 33%), and 7 ± 3 uU/ml (48% ± 34%), respectively.

At rest, GIP reached peak concentration in response to all beverages 15 min after beverage consumption. At rest, WP, CAS, and CAS-WP baseline GIP concentrations, with peak concentration in parentheses, were 32 ± 13 (207% ± 106%), 41 ± 13 (211% ± 100%), and 35 ± 13 pg/ml (179% ± 74%), respectively. Postexercise, GIP reached peak concentration in response to CAS, WP, and CAS-WP 15, 30, and 45 min, respectively, after consuming the beverages. Postexercise, WP, CAS, and CAS-WP baseline GIP concentrations, with peak concentration in parentheses, were 40 ± 22 (246% ± 87%), 51 ± 35 (237% ± 80%), and 67 ± 63 pg/ml (294% ± 90%), respectively. Although time to peak was different among beverages postexercise, there were no significant differences between AUCs ($p > .05$).

Insulinogenic Index

Results indicated that there was no significant beverage-by-time interaction at early time points (i.e., within the first 30 min after consuming the beverages) at rest or postexercise for this outcome. The insulinogenic indexes for WP, CAS, and CAS-WP at rest were 0.02 ± 0.01, 0.03 ± 0.02, and 0.03 ± 0.01, respectively, and postexercise were 0.40 ± 0.14, 0.47 ± 0.14, and 0.42 ± 0.19, respectively.

AA Response

Figures 4 and 5 present the postprandial responses at rest and postexercise for total AA (TAA) concentration (i.e., alanine, arginine, glutamine, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, serine, threonine, tyrosine, and valine), branched-chain AA (BCAA) concentration (i.e., valine, leucine, and isoleucine), and gluconeogenic AA (GAA) concentration (i.e., alanine, glutamine,

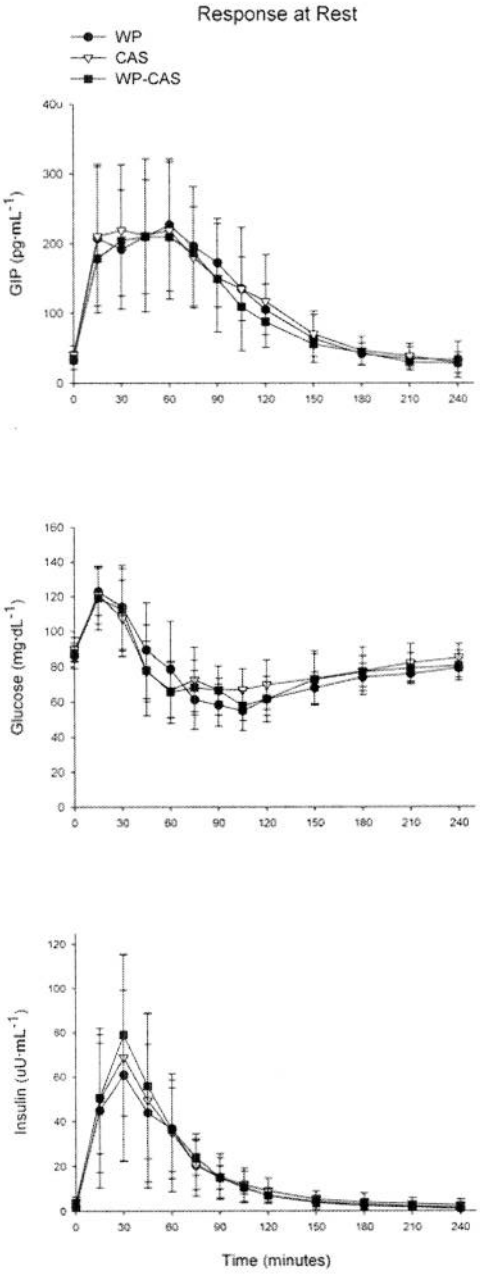

Figure 2 — Postprandial glucose, insulin, and GIP responses at rest. *Note*. WP = whey; CAS = casein; CAS-WP = casein and whey blend; GIP = glucose-dependent insulinotrophic polypeptide.

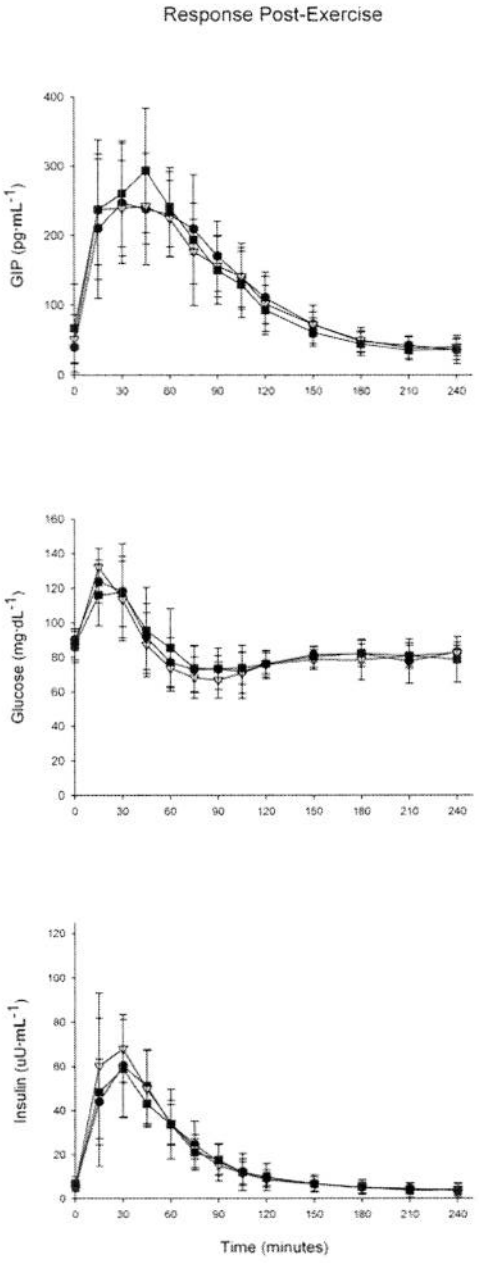

Figure 3 — Postprandial glucose, insulin, and glucose-dependent insulinotrophic polypeptide (GIP) responses postexercise.

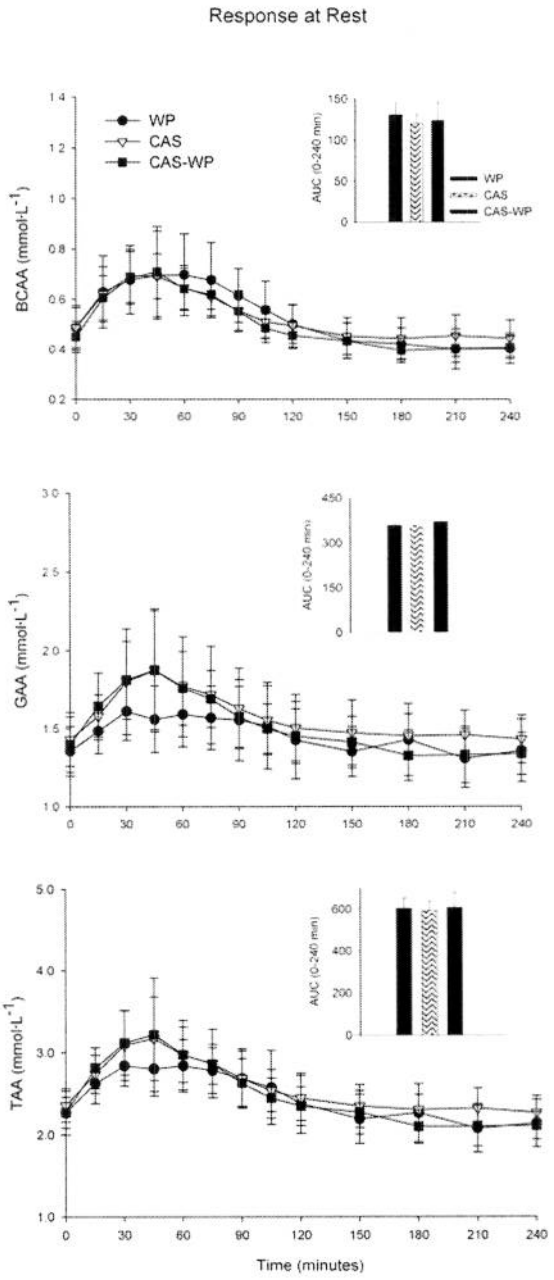

Figure 4 — Postprandial BCAA, GAA, and TAA responses at rest (with area under the curve [AUC] of 0–240 min inset). *Note*. WP = whey; CAS = casein; CAS-WP = casein and whey blend; BCAA = branched-chain amino acids; GAA = gluconeogenic amino acids; TAA = total amino acids.

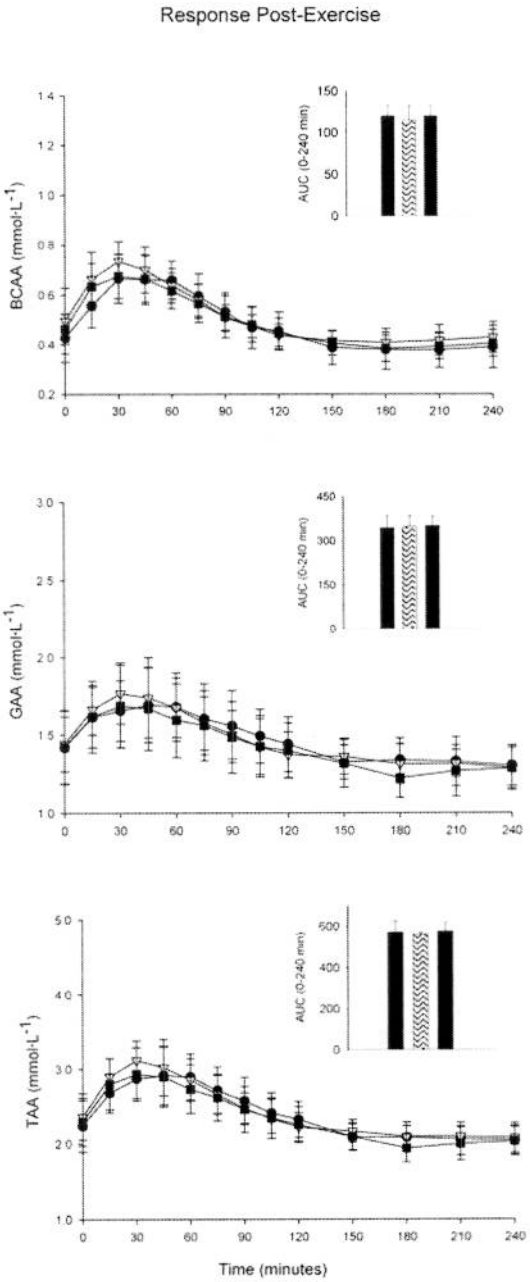

Figure 5 — Postprandial BCAA, GAA, and TAA responses postexercise (with area under the curve [AUC] of 0–240 min inset). *Note.* BCAA = branched-chain amino acids; GAA = gluconeogenic amino acids; TAA = total amino acids.

glycine, serine, and threonine), as well as AUC_G from 0 to 240 min (Figures 4 and 5 inset). At rest and postexercise, BCAA peaked 30 min after beverage consumption for WP, CAS, and CAS-WP (rest: 0.68 ± 0.14, 0.69 ± 0.11, and 0.69 ± 0.11 mmol/L, respectively; postexercise: 0.67 ± .03, 0.74 ± 0.08, and 0.68 ± 0.09 mmol/L, respectively). This corresponded to 40% ± 20%, 44% ± 25%, and 53% ± 21% increases from baseline at rest, respectively, and 60% ± 33%, 53% ± 25%, and 47% ± 18% increases from baseline postexercise, respectively. TAA, BCAA, and GAA increased ($p < .001$) after ingestion of WP, CAS, and CAS-WP both at rest and postexercise. Post hoc testing revealed, however, that there was no significant beverage-by-time interaction for TAA, BCAA, or GAA AUC_G 0–60 min or 0–240 min after beverage ingestion, regardless of whether the beverage was consumed at rest or postexercise.

Discussion

The primary purpose of this study was to determine whether incorporating different protein types into high-carbohydrate beverages, similar to those available to consumers, affects plasma AA profiles and hormonal responses and whether a blend of casein and whey, in a combination similar to milk, would reproduce the unique AA-absorption pattern of milk. To account for possible interaction with aerobic exercise, we tested the beverages at rest and during recovery from exercise. We hypothesized that we would observe a different AA profile in response to WP and CAS—that is, a faster, transient increase after consuming WP and a lower, more prolonged increase after consuming CAS—as a result of slower digestion of casein (Boirie et al., 1997; Dangin et al., 2001). In terms of hormonal response, we anticipated that WP would elicit a higher insulin and GIP response than CAS as demonstrated by Nilsson et al. (2004). In addition, we expected that CAS-WP would demonstrate a modified response, similar to milk, independent of exercise. Instead, we found no difference among carbohydrate-protein beverages in time course, time to peak concentration, or AUC for AA response, as well as similar insulin and insulinogenic index responses. These findings suggest that there were no differences in digestion and absorption between drinks, despite different milk-protein profiles. Possible explanations for the absence of any differences might be related to the physical state of the protein (i.e., partial hydrolysis) in CAS and CAS-WP, proportion of milk proteins within CAS and CAS-WP, or the amount of carbohydrate in the beverages.

Post hoc chemical analysis of the drink powders was conducted to determine whether partial hydrolysis of casein could have confounded the experiment. The analysis revealed that most of the protein in CAS and CAS-WP was intact (57% and 66%, respectively), but a significant portion (43% and 34%, respectively) was hydrolyzed. If we make a conservative assumption that all the hydrolysis in CAS is attributable to casein, then 56% of the casein in CAS was hydrolyzed and 44% was intact. If these estimated values are applied to the other beverages, the resulting predicted values of hydrolyzed protein content are comparable to the measured values of hydrolyzed protein content derived via chemical analysis (WP predicted = 4%, WP observed = 6%; CAS-WP predicted = 38%, CAS-WP observed = 34%). Dangin et al. (2001) and Silk et al. (1979) demonstrated that free AAs mimicking casein's AA profile are digested quickly and produce a

plasma AA response similar to that of whey protein, and Calbet and Holst (2004) reported that casein hydrolysates (vs. fully intact casein) produce more rapid insulin and AA responses. This is consistent with our results, in that our casein-containing beverages behaved similarly to WP at both initial and latter time points, and we observed higher peak BCAA concentration for CAS than previous research investigating intact casein (Lacroix et al., 2006; Nilsson et al., 2004). Because we did not observe any differences between the beverages in regard to AA (during either resting or postexercise condition) and hormonal response (i.e., during resting conditions), it would appear that the presumed partial hydrolysis of casein in our test beverages was enough to accelerate digestion and absorption and thereby produce drinks with similar physiological effects.

To address our experimental question, we developed a chocolate beverage similar to those available in the marketplace, using cocoa powder as the main flavoring ingredient, and manipulated the ratio of casein and whey. We acknowledge that there is some casein in WP and some whey in CAS, which reduces the proportion of protein contributed by whey protein in WP and casein in CAS. Because milk (80% casein) is distinguishable from a beverage with 100% whey protein (Lacroix et al., 2006; Nilsson et al., 2004), however, it stands to reason that the amount of casein in CAS (77%) should have been enough to distinguish it from the beverage containing 70% whey protein (WP). Furthermore, because our hormonal and AA responses to WP are similar to those reported in the literature (Lacroix et al.; Nilsson, Holst, & Bjorck, 2007), it does not appear as though the amount of casein in WP (2%) slowed digestion. Finally, the addition of cocoa powder to CAS might have accelerated digestion of this beverage, causing an AA response more similar to WP and making it harder to distinguish CAS from WP. There is evidence suggesting that cocoa powder stimulates a postprandial insulin response (Brand-Miller, Holt, de Jong, & Petocz, 2003), which in turn is strongly correlated to postprandial aminoacidemia (van Loon et al., 2000). Cocoa powder alone, however, is unlikely to be the primary factor affecting our inability to detect differences between CAS and WP, because only a relatively small amount of the protein content in CAS was contributed by cocoa powder (approximately one fifth).

It has been suggested that ingesting carbohydrate with protein attenuates the AA response compared with consuming protein alone (Dangin et al., 2002). Although we reported a lower peak leucine response to WP (2.4 mmol/L) than did Boirie et al. (1997) and Dangin et al. (2001), who fed protein only (3.5 and 4.0 mmol/L, respectively), AA responses to WP in this study are similar to those of others who fed carbohydrates with protein (Lacroix et al., 2006; Nilsson et al., 2007). For example, peak BCAA response to WP in this study was similar to that reported by Lacroix et al. (0.70 vs. 0.78 mmol/L, respectively), who fed carbohydrates (35 g) and whey protein (23 g) in a 1.5:1 ratio. Furthermore, peak leucine response to WP in this study was higher than that reported by Nilsson et al. (2007; 0.24 vs. 0.15 mmol/L, respectively), who fed carbohydrates (25 g) and whey protein (18 g) in a 1.4:1 ratio. Therefore, it does not appear that adding more carbohydrate to the beverages further blunted the AA response. Moreover, the additional carbohydrate seems unlikely to have interfered with the ability to detect differences between beverage formulations.

Although the GIP response was consistent between beverages at rest, we detected differences in time to peak between the beverages when they were

consumed during recovery from exercise. The differences in time to peak did not, however, affect the AUCs—no significant differences between beverages were identified. Although there was no significant beverage-by-time interaction at early time points, the insulinogenic index values during recovery from exercise suggest that insulin response postexercise was driven by factors other than glucose and reveal a unique difference between resting and postexercise. The variable time to peak GIP response after exercise confirms the importance of comparing beverages under resting and postexercise conditions if beverage formulations or foods are designed to be consumed at rest and during postexercise recovery.

We aimed to test how varying contents of casein and whey in carbohydrate-protein beverages affected aminoacidemia and insulinemia. We propose that we observed little difference in these variables because of the partially hydrolyzed nature of the casein protein we obtained for the experiment. Our results have practical implications for consumers who purchase these beverages for use at rest or during recovery from exercise. Because our protein sources were purchased from a major supplier to the commercial industry, it is probable that manufacturers of these types of beverages purchase their protein powder from the same source (or another supplier that is unaware that its protein powder is partially hydrolyzed). Therefore, consumers should be aware that these products might not contain the specific protein sources being advertised.

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